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Alice Jesus José Brás

Melatonin: a shining bridge between chronodisruption and type 2 diabetes

Melatonina: uma ponte em evidência entre cronodisrupção e diabetes tipo 2

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Aos meus pais,

Melatonin: a shining bridge between chronodisruption and type 2 diabetes

Melatonin, chronodisruption and type 2 diabetes

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KEYWORDS: melatonin; chronodisruption; circadian rhythm; oxidative stress; shift work; circadian misalignment; diabetes; obesity.

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ABSTRACT: Melatonin (ML), a key hormone in chronobiology, is released from pineal gland obeying a circadian rhythm, and exerts many of its actions through MT1 and MT2 receptors. The circadian clock, in face of nowadays demands of irregular schedules and technologies pressure, is forced to misalignment, a consequence related with different chronic diseases, as obesity and type 2 diabetes mellitus (T2DM). On the other hand, sleep disorders, featured by circadian disorders, can result from diabetic conditions. We review data showing that disturbed circadian rhythms may result in glucose intolerance, T2DM and other metabolic disorders. Here, we praise melatonin as a link between chronodisruption and diabetes and a potential adjuvant for its treatment. We first describe melatonin physiological role on circadian system, the involvement of chronodisruption factors in diabetes pathogenesis, to later focus on the beneficial role of melatonin in this condition. Lastly, we summarize current knowledge about the therapeutic properties of melatonin in diabetes.

INTRODUCTION

Isolated by Aaron Lerner in 1958 from bovine pineal glands (1), Melatonin (ML), an indoleamine with the chemical name 5-methoxy-N-acetyltryptamine (2), is discharged from epiphysis or pineal gland in a pulsatile manner, in consonance with light-dark cycle and daily and seasonal changes (3). ML shows a circadian rhythm controlled by circadian pacemaker cells, the "master circadian clock" on suprachiasmatic nucleus (SCN), situated in the ventral part of the hypothalamus (4). In mammals, its high levels in plasma during night was found to be 80-100 pg/mL and low levels during day of 10-20 pg/mL (5). ML plasma hormone profile reproduces exactly pineal activity, since ML is not stored in the pineal gland (5). It is also released by other organs or cells, but in less amount or due to a specific stimulis (6).

ML synthesis occurs according the following steps: tryptophan (Trp), the synthetic precursor, is metabolized into 5-hydroxy-Trp (5-HTP) by Trp-hydroxylase (TPOH), which is then converted into 5-HT by an aromatic amino acid decarboxylase (AAAD). 5-HT is first acetylated by arylalkylamine-N-acetyltransferase (AA-NAT) into N-acetylserotonin (NAS), and finally O-methylated by HIOMT to produce ML (5).

ML, mostly released from pineal gland during night, activate MT1 and MT2 receptors by a feedback mechanism on SCN, leading to a phase shift in local and biologic circadian rhythms (3). In fact, ML is a key molecule in chronobiology and is the most important efferent hormonal signal acting as an internal "zeitgeber" (7), either by resetting feedback signal to SCN or by timing other cells in central nervous system (CNS) and peripherally (6). Thus, ML indicates the time of the day to peripheral organs and tissues (7), regulating the rhythmicity expression of "clock genes" at a cellular level (8) in order to adjust their metabolic activity (7) (Figure 1). ML also has specific actions in sleep induction, vasomotor regulation and adrenal activity, along with anti-excitatory actions, anti-inflammatory properties, antioxidant actions and energy metabolism modulation (6).

Human clock is able to maintain 24-h rhythms, even in the absence of any environmental cues. However, it uses these environmental signals to synchronize their endogenous rhythms, called "zeitgeber", meaning time givers, of which the most important neuronal signal is light (9). In mammals, after light detection by retinal photoreceptors, action potentials are transferred to the SCN via the retinohypothalamic tract (10). The paraventricular nucleus of the hypothalamus projects to the preganglionic

neurons at the intermediolateral column of the upper thoracic cord. After synapsing with these cells, the preganglionic axons go on to the rostral third of the superior cervical ganglia, sending postganglionic sympathetic projections to pinealocytes, the endocrine elements of the pineal gland (10). During darkness, the SCN sends neural impulses which induce norepinephrine discharge from the postganglionic terminals near the pinealocytes, leading to the nightly rise in pineal melatonin synthesis (11). These neurological connections occur in a different way when light is present during night, suppressing ML production and declining its circulating levels (12) which can negatively impair metabolic functions (13). Additional "zeitgebers" as feeding, physical activities, social environment and biological stress also contribute to modulate circadian rhythm (14-16). Simultaneously, the master circadian clock integrates information from epigenetic factors and synchronizes oscillators on peripheral tissues by controlling about 10-20% of cellular genes (16).

Therefore, mammalian cell-autonomous molecular clock is generated in 24 – hour rhythm by multiples interlocking molecular loops located in almost every cell, involving rhythmic transcription of specific "clock genes" and interactions of the encoded proteins, organized hierarchically by the "master clock" (17, 18). In the last decade, the clock mechanism was first described as a simple transcription-translation feedback loop (TTFL) driven by four integral clock proteins and including a positive and a negative limb. In this way, BMAL1 (brain and muscle ARNT-like 1) and CLOCK (circadian locomotor output cycles kaput), components of the positive limb, dimerize and bind to E-box-containing DNA elements and, consequently, activate the transcription of cryptochrome (CRY1 and CRY2) and period genes (PER1 and PER2). CRY and PER are repressors genes, that belong to the negative limb, inhibit the function of CLOCK/BMAL1 (17, 18). PER and CRY complexes are degraded through E3 ubiquitin ligase pathways, reliving the repression of CLOCK/BMAL1, and a new ~24h cycle begins (18, 19). Along with this loop, casein kinases, CKIε and CKIδ, regulated by phosphatases, PP1 and PP5, control the activity of Per/Cry complexes (18).

After this time, multi-loop oscillators have been introduced by discovering additional clock genes. These included a second TTFL activated by retinoid-related orphan receptors (RORα, RORβ and RORγ) and repressed by REV–ERBα (encoded by NR1D1) and REV–ERBβ (encoded by NR1D2) (18, 19). Another interlocking transcriptional feedback loop involves the PAR-bZip (proline and acidic amino acid-rich basic leucine zipper) transcription factor family, including DBP (D-box binding protein), TEF (thyrotroph embryonic factor) and HLF (hepatic leukaemia factor) (19).

These molecular feedback loops, expressed in multiple tissues in the body, are able to coordinate rhythmic processes in each cell (20). However, clock genes polymorphisms variants may lead to metabolic disorders as dyslipidemia, diabetes, food intake and gestational diabetes (21). Likewise, inappropriate cues may result in central clock misalignment and consequently cellular clock genes machinery malfunctioning, leading to circadian disruption (chronodisruption) (11) and metabolic conditions as diabetes and obesity (22).

MELATONIN RECEPTORS

Melatonin is released both from pineal gland and retina and many of its actions are mediated through interaction with two high-affinity G protein-coupled receptors (3). Melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2) are the main two types melatonin receptors, both typically coupled to $G_{i/o}$- type proteins (23). These receptors show distinct chromosomal sites, MT1 with 4q35.1 for MTNR1A gene and MT2 with 11q21-q22 for MTNR1B gene (23). The MT1 melatonin receptors couple to both pertussis toxin (PTX) sensitive G_i and insensitive $G_{q/11}$ G proteins. Binding to these receptors results in a decrease of forskolin-stimulated cAMP formation and inhibition of both protein kinase A activity and phosphorylation of the cAMP responsive element-binding protein (CREB). Additionally, ML, through MT1, can potentiate conductance of inward-rectifier potassium channels (Kir), stimulate phosphorylation of mitogen-activated protein kinase 1/2 (MEK1/2) and extracellular signal-regulated kinase 1/2 (ERK1/2). Regarding MT2 melatonin receptor signaling, its activation inhibits forskolin-stimulated cAMP and cGMP formation. In the SCN, MT2 melatonin receptors activate protein kinase C (PKC), while in retina reduce neurotransmitter release through calcium-dependent mechanisms (24).

A high degree of sequence homology is shared by MT1 and MT2 receptors (23). Melatonin bind to both MT1 and MT2 receptors with high affinity being methoxy group and acetamide side chain responsible for, respectively, ML intrinsic activity and binding affinity in humans (23). Curiously, there are simultaneously additive, synergistic, or opposing responses when activation of both MT1 and MT2 melatonin receptors in the same or in different cells (3). In fact, human MT1 and MT2 receptors can form homo- and hetero-oligomers between themselves and other G protein–coupled receptors, considering that MT1/MT2 heterodimers formation is 3- to 4-fold lower than the formation of MT1/MT1 homodimers and is similar to the formation of MT2/MT2 homodimers (25). ML receptor heteroligomers have an essential role in retinal function, once MT1/MT2 heterodimers in mouse rod photoreceptors modulate light sensitivity via a phospholipase C (PLC)/PKC pathway, which is not normally triggered by activation of monomeric units (26). MT1 and MT2 receptors are also able to heterodimerize with the serotonin receptor (5-HT2C) (36), which is particular relevant concerning agomelatine, a ML receptor agonist that exerts antidepressive effects by 5-HT2C receptor antagonism (27).

ML can also bind to a third ML receptor, previously defined as MT3, a member of the quinone reductases family (23). Additionally, ML also exerts indirectly its effects through nuclear receptors, binding to a retinoid-related orphan nuclear hormone receptor from the RZR/RORα family, a member of transcription factors (28) and through direct interactions with intracellular proteins, including calmodulin, calreticulin and tubulin (29). The highest expression of ML receptors has been found in the pars tuberalis of the anterior pituitary (30), and also in quite a number of sites in the CNS (31). Likewise, more membrane receptors have been found in peripheral organs, such as in gastrointestinal tract, in gallbladder epithelium, parotid gland, exocrine and β-cells of endocrine pancreas, skin, breast, uterus, fetal kidney, cardiac ventricular wall, central and peripheral vasculature aorta, brown and white adipose tissues, platelets, immune cells, cancer cell lines (6).

WHEN ARTIFICAL LIGHT CHANGES OUR NATURAL PATTERNS

A wide diversity of physiological and behavioral circadian rhythms are controlled by internal biological clocks, predominantly synchronized by the environmental light/dark cycle (12). In face of social and economic demands in industrialized countries, people became trapped in some type of permanent night and rotating shift work, and also susceptible to "jet lag" due to travels across several time zones (32). Consequently, these type of works mean irregular schedules and forced exposure to nocturnal lighting, showing significant disruptions in sleep architecture and other markers of circadian synchronization (33). Sleep disruption is also a core feature of posttraumatic stress disorder resulting in circadian disruption (34). A "social jet lag" also has been linked to circadian misalignment, due to changes in sleep behavior, as shorter sleep duration and later chronotype (35). Simultaneously, there was a rise of exposure to light during night related with increased time of wakefulness, use of artificial light, television, computer monitors and other technologies (29) that disrupt the natural cycle of sleep/wakefulness, exposing human organism to light at atypical biologic times (32). Curiously, nocturnal blue light exposure presently growing due to the proliferation of energy-efficient lighting (LEDs) and electronic devices appears to induce the strongest ML inhibition and cause more disturbances in clock balance at night. It was also suggested that development of lighting systems maintaining the natural ML rhythm could decrease these effects (36). Caffeine (37) and alcohol (38) might also contribute to cause phase shift in circadian pacemaker time.

CONSEQUENCES OF CHRONODISRUPTION

Artificial light during night has negative chronobiologic effects (32), suppressing ML synthesis and secretion in different species, including humans (12). Accordingly, alterations in ML production result in an imbalance of biological rhythms, converging to marked misalignment of the central circadian pacemaker. Consequently, the result is the disorganization of peripheral cellular clocks and malfunctioning at tissue, molecular and cellular levels (32) (Figure 2).

Circadian disruption reflects a desynchronization either among endogenous circadian (~24-hour) rhythms or between them and the environment with consequences to cardiometabolic health (39). Considering ML essential to optimize energy balance and metabolism, there is increasing evidence that disruption of normal circadian rhythms may lead to metabolic circadian disorders, namely glucose intolerance, insulin resistance and obesity (22) as also metabolic syndrome (40, 41). Light-dark misalignment might be able to accelerate weight gain, in both animals and humans, not only due to changes in ML patterns, but also in eating behavior, hormones and in response to stress (42). However, obesity and other metabolic diseases might be able to disturb normal clock timing and amplitude in multiple organ systems, and consequently exacerbate disease progression (43). Therefore, other lipid metabolic conditions have been related to chronodisruption (44). On a cardiovascular view, shift work was associated with an increased risk of coronary disease, acute myocardial infarctions and cerebrovascular accidents, even after adjusting for possible confounds (45). Circadian misalignment is also a factor to increase the risk of developing cancer, particularly breast (46), colon (47) and prostate cancer (48).

DIABETES – AN EMERGENT PROBLEM LINKED TO CIRCADIAN MISALIGNMENT

Diabetes is becoming a global problem, affecting around 387 million people worldwide, 90% with type 2 diabetes, and with an expectable double increase in prevalence over the next 20 years (49). This prevalence increase is related with nowadays lifestyle, featuring by excessive food consumption, sedentary life style and lack of physical activity (49), ultimately leading to glucose homeostasis disruption (50). In addition to pancreatic β-cell dysfunction, insulin resistance, abnormal adipogenesis and gluconeogenesis increase play an important role in T2DM progression (49). However, disruption of circadian rhythm mechanisms and genetic susceptibility has also been implicated in the development of T2DM (51). Circadian misalignment also reflects itself in timing disruption of food intake and biological functions, sleep disorders, changes in ML signaling pathways and clock gene expression and presence of polymorphisms. Altogether, these consequences have also been attributed to diabetes pathogenesis (51).

Although people with primary sleep disorders have been strongly associated with increased T2DM, diabetes may also lead to disruption in sleep quality, as a result of thirst at night, nocturia, polyuria, diabetic neuropathy and associated pain, symptoms of hypoglycemia, and also due to associated chronic illness as obstructive sleep apnea, hypertension and cardiovascular complications, cerebrovascular accidents and depression (52).

Epidemiologic studies have shown an association between shift work and risk of developing TDM2, while ML supplements have been used to treat insomnia or jet lag (53). Additionally, evening light exposure, associated with changes in timing and amount of ML secretion, expression of clock genes and food intake, is suggested to be more correlated with diabetes risk than light exposure in other periods of time, though these consequences were proposed to be independent of changes in nocturnal ML levels (54).

MELATONIN RECEPTORS AND DIABETES

ML can influence either directly clock machinery in pancreas or indirectly via the SCN (55). Molecular and immunocytochemical studies confirmed the existence of MT1 and MT2 receptors in Langerhans islets and in human pancreatic tissue, in which ML could exert its effects to maintain circadian rhythms and consequently glucose homeostasis (55). The possible association between ML receptors and T2DM pathogenesis has been investigated using ML receptor knockout (KO) mice to determine the mechanisms by which these receptors could contribute to the regulation of glucose homeostasis and insulin sensitivity. MT1 receptor silencing showed higher mean blood glucose levels comparing to controls (56), as well as significantly impaired glucose metabolism and insulin resistance comparing with wild type (WT) and MT2-/- mice (57). Moreover, MT1 -/- and MT2 -/- mice abolished the daily rhythm in blood glucose levels (58). Pinealectomy in rats, by abolishing endogenous ML levels, altered the daily rhythm in blood glucose levels with higher levels during the night (59).

Additional growing evidence has shown a clinical relationship between ML receptors variants and impaired insulin secretion, ineffective glucose metabolism (60, 61), increased risk of T2DM (62) and gestational diabetes mellitus (63). Several large

genome-wide association studies have identified more than 150 risk alleles for T2DM, most of them affecting genes that encode intracellular proteins mediating insulin secretion by β-cells or transducing the effects of insulin in target tissues, responsible to increase individual susceptibility to diabetes (53). In this context, common single nucleotides polymorphisms (SNP), as rs1387153 and rs10830963, were identified located near the gene MTNR1B that encodes the MT2 receptor of ML (62). Among these SNP, those causing loss of ML receptor function were associated with the highest incidence of T2DM. The SNP rs10830963, the variant with the strongest association signal, was related to impairment of early insulin secretion and β-cell dysfunction, which might be responsible for increasing the T2DM risk (62).

ML during the night is released from pineal gland and binds to MT2 receptors, leading to a reduction of ATP conversion to cyclic AMP (cAMP) and consequently reduction of protein kinase A activation. This pathway ultimately results in the inhibition of insulin secretion in response to glucose metabolism. However, when MT2 receptor variants associated with diabetes risk are present, MT2 receptors are up-regulated in islet β-cells leading to a higher inhibition of cAMP formation and consequently a stronger inhibition of glucose-stimulated insulin secretion (53).

GLUCOSE HOMEOSTASIS AND MELATONIN

Growing evidence of an interplay between ML and diabetes have been increasing (64), supporting that loss of nocturnal ML secretion is associated with a higher risk of developing T2DM (65). In fact, pinealectomy and sympathetic denervation of the upper sympathetic ganglia lead to a reduction in ML secretion, and, on the other hand, increased food consumption and weight gain (66). Removal of the endogenous source of ML in rats was also associated with a lower insulin response, with glucose intolerance and a decrease in Glut4 expression in both adipose and muscle tissues (67). Furthermore, it also decreased hepatic and muscular glycogenesis and caused an increase in blood pyruvate concentrations (66).

At a cellular level, ML treatment also showed improvements in glucose homeostasis by stimulating glucose transport in skeletal muscle via phosphorylation and activation of insulin receptor substrate 1 (IRS-1) and phosphotidyl-inosytol 3 kinase (PI-3K), and hence restoring the vascular actions of insulin and stimulating glycogen synthesis in hepatic cells, respectively (68). In vitro studies with human pancreatic islet cells suggested that ML has direct stimulatory effects in β-cells by increasing insulin secretion and improving glucose sensitivity (69, 70). Furthermore, persistent activation of ML signaling in vitro had the potential to attenuate β-cell loss and dysfunction associated with molecular stress present in T2DM (71). This observation was explained by an activation of the cAMP-dependent signal transduction pathway and decrease of toxicity β-cell apoptosis due to a reduction of caspase-3 cleavage, decrease on stress-activated protein kinase/Jun-amino-terminal kinase activation and attenuation on oxidative stress response (71).

Evidences of ML regulation in human metabolic balance have been emerging. A hypothetical antagonism interplay between insulin and ML was evident in a cross-sectional study enrolling 1,075 women without diabetes, hypertension, or malignancy, that found that higher nocturnal ML secretion was independently and inversely associated with insulin levels and insulin resistance (72). A case-control study among women participating in the Nurses' Health study found an association between ML secretion and risk of developing T2DM, independently of established diabetes risk

factors, and that non-diabetic individuals with low ML secretion during night showed an increased risk of developing diabetes (65). Another study showed that nighttime ML level was significantly lower among subjects with proliferative diabetic retinopathy, compared with healthy subjects, a finding suggested to be due to advanced dysfunction of retinal light perception, and consequently ML secretion alterations (73).

MELATONIN AND INSULIN

Insulin secretion, similarly to ML, shows a circadian pattern in humans, revealing an increase from a low point between midnight and 6 AM and reaching a peak between noon and 6 PM (74). A functional antagonism between insulin and ML might exist as at night insulin levels are low and ML levels elevated, whereas during the day insulin levels are high and ML low (75). ML improves insulin signalization both in CNS and peripheral tissues, and insulin potentiate an elevation of ML secretion at night (64). The circadian clock is able to induce variations in insulin sensitivity in T2DM patients (51), that present lower circulating levels of ML, comparing to healthy individuals (76). Pineal ML synthesis has been reported to be decreased in type 2 diabetic Goto-Kakizaki (GK) rats, compared with non-diabetic Wistar rats (77), and local hyperglycemia can be involved in this pathogenesis (64). These differences in diabetic rats were attributed to a different expression pattern of ML synthesizing enzymes, less amount of its precursors, and less noradrenaline levels in pineal glands (77). Attending to the role of catecholamines in decreasing insulin secretion and stimulating ML synthesis, it was observed that noradrenaline, adrenaline and ML levels were reduced in type 2 diabetic GK rats, that are characterized by high insulin levels (78). Further, decreased pineal gland size and up-regulation of inhibitory β-adrenoceptors (71) were observed in animal models of T2DM.

MT1 and MT2 receptors, involved in insulin secretion modulation, are both expressed in Langerhans islet (55) and their desynchronization signaling pathways may be involved in diabetes pathogenesis. ML influences insulin secretion by MT1 and MT2-receptor-mediated effects (69). There are divergence results on receptor function, since some authors observed an increase in insulin secretion from isolated human islets mediated by ML (70), while others reported a reduction of insulin secretion in rodents islets (69, 79, 80). In isolated pancreatic islets of neonate rats and INS-1 cells, ML induced insulin secretion through forskolin-stimulated cAMP production, an activator of adenylate cyclase (AC) activity (69, 79, 80). According, luzindole, a competitive ML receptor antagonist, is able to mostly reverse this inhibitory effect of adenylcyclase/Camp on insulin production (79). These findings indicate the potential inhibitory influence of ML on pancreatic β-cells by cAMP-signaling pathway via MT1 receptors. ML actions extend to other molecular mechanisms as the inhibition of cyclic guanosine 3',5'-monophosphate (cGMP) signaling pathway mediated by MT2 receptor consequently suppress insulin secretion (81). The circadian clock genes probably are modulated by cGMP, via protein kinase G (PKG), leading to a phase shift and resetting of secretion rhythms (55). In INS-1 cell line, ML seemed also to have a dose-dependent stimulation of 1,4,5-triphophate (IP3) increasing effect, resulting in insulin release mediated by intracellular calcium mobilization (82).

Furthermore, ML arises as an important hormone to improve β-cell survival, attenuation of β-cell turnover and β-cell function enhancement (83). This regulation can be explained by the cAMP-dependent signal transduction pathway involving PKA,

essential for the stimulation of acute insulin response to glucose, and CREB activation, critical to maintain adequate β-cell mass, function and protection against B-cell apoptosis (71).

CHRONODIRUPTION AND DIABETES

Several lines of evidence have correlated circadian disruption and impairment of glucose balance (Figure 3). A human experimental study evaluated the adverse cardiometabolic implications of circadian misalignment, as occurs acutely with jet lag and chronically with shift work, showing systematic increases in postprandial glucose, insulin and mean arterial pressure (84). Shift workers, under the circumstances of irregular sleep schedules and sleep deprivation, show an increased risk of diabetes relatively to day workers (85), though not achieving statistical significance. By other side, patients with diabetes presented sleep disorders and rhythmic disturbances (86). Additionally, as occur in shift work, induced sleep restriction showed a significant decrease in insulin sensitivity and an increase in low-grade inflammation (87), known to have a potential role in diabetes, obesity and metabolic disorders (88). Short sleep duration, sleep quality and long sleep duration have been connected to an increasing risk of developing T2DM (89). Men reporting ≤5 and 6 h of sleep per night were twice as likely to develop diabetes, as those who reported sleeping 7 hours per night. Long sleep duration also increased the risk of developing diabetes (90). Similarly, healthy adults exposed to prolonged sleep restriction, with concurrent circadian disruption, had significantly decreased resting metabolic rate and increased postprandial plasma glucose, via inadequate pancreatic β-cell responsivity (91). Circadian misalignment experimentally induced in healthy subjects showed a decrease in leptin concentrations (84). Leptin, synthetized by adipocytes, inhibits food intake and influence peripheral metabolism of glucose and lipids (92). Since reduced levels of leptin can stimulate appetite and distort energy expenditure (84), it may provide a mechanism underlying the increased risk for obesity and diabetes. In addition, circadian misalignment resulted in a complete inversion of cortisol profile that similarly can be associated with insulin resistance and hyperglycemia (84). As cortisol is an important regulator of peripheral clocks, cortisol rhythm desynchronization may have an impact on metabolic homeostasis (92). Furthermore, glucose intolerance is associated with high circulating cortisol concentrations, which is responsible for raising blood glucose levels through different mechanisms (93).
Glucagon also has an important role in blood glucose homeostasis, and total sleep loss was shown to lead to a decrease in glucagon levels in humans (94).

These data are in concordance with results obtained from animal studies. Disturbance of circadian rhythms due to shift work, sleep loss, or nocturnal lifestyle were simulated by experimental circadian rhythms disruption in diabetes-prone HIP rats, showing a rapid manifestation of T2DM, due to accelerated loss of β-cell function and mass attributed to the increase of β-cell apoptosis (95). A recent study showed that combination of ML and metformin in rats concomitantly exposed to circadian disruption and diet-induced obesity, synergistically change the progression of metabolic dysfunction through improvement of adiposity, circadian activity, insulin sensitivity and islet cell failure (83).

CHRONODISRUPTION AND OBESITY – IMPLICATIONS IN T2DM

Chronodisruption and reduction in sleep duration over the last decades are conditions involved in obesity increase (96, 97). In turn, overweight and obesity are main risk factors for T2DM pathogenesis (98). Here we review some data of circadian disruption and its consequences in obesity, a mechanism that can be involved in diabetes pathogenesis. Disruption in central clock can decrease energy expenditure and consequently lead to obesity induction (99). Moreover, exposure to light at abnormal hours was associated with weight gain in rodents and with higher odds ratio for obesity in humans (14, 100). Brown adipose tissue (BAT) is positively related with a protective role in diabetes and lower glycaemia in humans (101). Prolonged light hours exposure lead to an increase in body fat mass through reduction of BAT activity and to a reduction of sympathetic stimulation of BAT and the β3-adrenergic intracellular signal, which consequently reduced the uptake of fatty acids from triglyceride-rich proteins and of glucose by BAT (99). Adipose tissue secretes molecules that modulate food intake, metabolic activity and fat deposition. Consequently, disturbances in these functions, could lead to metabolic dysregulation, influencing the timing of food intake and fat deposition (97). Moreover, some genes in adipocytes exhibit circadian rhythms and disturbances in central or peripheral circadian clock can have impact on normal functioning of adipocyte genetic machinery (97). Circadian clock gene network perturbations play an important role in metabolic balance dysregulation, leading to alterations of food intake rhythm, hyperphagia, hyperlipidemia and hepatic steatosis (102). Deficiency of ML, observed in sleep deprivation, has been demonstrated to be correlated with obesity (96), and ML supplementation might have beneficial effects on obesity and its complications (103). Furthermore, BAT was suggested to be a potential target for novel anti-obesity drug development, seeming to be a factor whereby animals lose weight in response to ML administration (36). Additionally, leptin and adiponectin, adipokines with an important role in glucose and lipid homeostasis, can have their circadian rhythms altered in obesity, and ML has been found to normalize the expression and secretion patterns of both adipokines (29).

Further studies have shown disruption of the circadian clock, either via dietary restriction or phase shifting, could affect rhythms within the gut microbiome constituents (104). Recently, it was suggested that human microbiome may affect the risk of obesity and T2DM, and that modulation of microbiome might reduce that risk (105). As ML, present at high levels in the gut, could have a plausible impact on this balance (104), we hypothesize whereas this hormone could play a plausible beneficial impact on this homeostasis.

OXIDATIVE STRESS DELETERIOUS EFFECTS ON DIABETES

Hyperglycemia causes a reduction in the levels of protective endogenous antioxidants and increases generation of free radicals, related with β-cell function impairment (106). Oxidative stress, one of the most harmful outcomes related to diabetes (64), may significantly decrease insulin cell signaling and glucose uptake by adipocytes (107), and attenuation of ML synthesis may aggravate this situation. Consequently, it could result in mitochondrial dysfunction and in cellular deleterious effects, which can be prejudicial for pancreas homeostasis, as pancreatic β-cells are

particularly susceptible to oxidative stress due to its low-antioxidative capacity (107). Additionally, ML parenteric administration was able to reduce the degree of lipoperoxidation and protein glycosylation (108). In vivo and in vitro studies showed ML attenuate oxidative stress in T2DM (13). In nondiabetic and T2DM human pancreatic islets, ML administration, mimicking a typical nightly exposure, showed attenuation of proteotoxicity-induced β-cell apoptosis evidenced by reduced caspase-3 cleavage, decreased activation of stress-activated protein kinase/Jun-amino-terminal kinase and diminished oxidative stress response (71). In T2DM non-insulin dependent, it was found, after 30-day ML supplementation, an improvement of antioxidative defense, suggesting ML supplementation as a beneficial additional treatment for controlling diabetic complications, especially considering the link between oxidative stress, aging, and diabetes type 2 (109).

Diabetic retinopathy is considered a major complication of T2DM, mainly due to the adverse effects of oxidative stress and inflammation (110). ML could protect against diabetic retinopathy, reducing some retinal histopathological changes in diabetic rats (111), by stimulation Akt-Nrf2-mediated antioxidant system and simultaneously inhibition NF-κB and its downstream proinflammatory cytokine production (112). Its protective functions were also mediated by reduced elevated VEGF expression, allowing reversion of retinal dysfunction due to DM (112).

Another complication of diabetes is neuropathy, with a prevalence rate of 50-60%, a condition in which ML also showed neuroprotective effects, mostly by attenuating NF-κB activation cascade and oxidative stress (113). Previous studies added to these evidences other protective roles of ML in renal, corneal and neural DM injuries (113-115).

Additionally, antioxidant properties of ML showed to be able to improve impaired functions of hepatic mitochondria in diabetic obese animals, thought to play a role in obesity and T2DM, in terms of development of liver steatosis and insulin resistance (116). In diabetic rats, ML also has the capacity to inhibit lipid peroxidation and regulate antioxidant enzymes, for which contribute its anti-apoptotic effects and antioxidant properties (117). The protection exerted by ML is mediated by directly scavenging free radicals (118) and stimulating the activity and expression of antioxidant enzymes, through the metabolization of free radicals and radical products to innocuous metabolites (119). The antioxidant effects of ML seem to rely on MT receptor-dependent and -independent mechanisms. ML binds to the MT3 binding site and to the cytosolic enzyme quinone reductase 2 (QR2), which in turn acts as a detoxifying enzyme to increase antioxidant defense (23). ML exhibits several unique features which differ from the classic antioxidants. In fact, ML and its metabolites are able to neutralize numerous toxic oxygen derivatives radicals (120). ML has the capacity to scavenge up to 10 reactive oxygen species (ROS) versus the classic antioxidants, being superior to vitamin C and vitamin E, under in vitro and in vivo conditions (120). So, ML has the ability to scavenge free radicals, known as the free radical scavenging cascade, but also to be induced under moderate oxidative stress (120).

Moreover, its antioxidative effects are potentiated by its capacity to activate cytoprotective enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, involved in metabolization of free radicals and radical products to innocuous metabolites (119). A further hypothesis was investigated concerning the fact that ML might influence receptor-mediated function by changing redox state at cellular level (23).

MELATONIN – IS IT AN OPTION TREATMENT?

Suppression of ML synthesis and secretion during night by nocturnal light abnormal exposure could be a critical factor for T2DM (121). As disturbed circadian rhythm, may be related to the T2DM pathogenesis, ML might be a useful option due to its ability to return both phase and amplitude of circadian clock (51). Chronic oral ML treatment showed reduction of liver steatosis and mitochondrial dysfunction, both enrolled in the development of insulin resistance in diabetic obese rats (122). It also decreased plasma glucose and hyperlipidemia levels (123) in obese rats, and showed a protective effect against the onset of diabetes (123). An increase in insulin secretion and a decrease in sensitivity of peripheral tissues and of pancreatic islet β-cells to insulin, a common pattern in aging, were counteracted with chronic ML in a murine model of accelerated aging (124). A long-term ML administration in Zucker diabetic fatty rats, as these beneficial effects on insulin sensitivity, have also been linked to body weight reduction and improved lipid metabolism (125). In male Wistar rats fed a high fructose diet, two weeks of ML administration also reduced insulin resistance and showed an increase in serum adiponectin levels and a reduction in leptin levels (126). Moreover, levels of circulating free fatty acids (FFA) and serum tumor necrosis factor (TNF-α), important factors implicated in the development of insulin resistance and diabetes type 2 (127), have also been reduced by ML treatment in rats (126). A study involving ZDF rats showed that ML administration during six weeks increased Ca^{2+} levels in liver, pancreas, muscle and in different adipose tissues (128), a probable mechanism of action of ML antidiabetic effects. This effect was explained by the way that increase in Ca^{2+} levels appears to improve insulin sensitivity, which may in turn be related to the elevation of adiponectin levels after ML administration (128). A recent study, using a metabolic syndrome rat model, showed that treatment with both low- and high- ramelteon doses attenuated cardiac injury and improved insulin signaling in white adipose tissue (129).

Evidences in humans have been providing ML treatment beneficial effects on T2DM patients, as well as on other related conditions. A placebo-controlled double-blind study, including 46 type 2 diabetic patients, reported that combination of zinc acetate and ML improved dyslipidemia and decreased the level of microalbuminuria (130). Other study using the same combination, both in monotherapy or together with metformin, showed improvement in glycemic control in T2DM (131). In insomnia patients with diabetes prolonged-release ML treatment during 5 months showed beneficial effects on glycated hemoglobin (HbA1c) (132). Additionally, administration of ramelteon to T2DM patients for 3 or 6 months did not change the level of HbA1c, however its discontinuation slightly increased the HbA1c level from 6.7% to 6.9% (133). Another study of isolated human islets from both healthy and T2DM donors had extended the evidence of ML crucial role in preservation of β-cell mass and function in T2DM patients, and revealed that in islets exposed to chronic hyperglycemia, ML could restore glucose-stimulated insulin secretion (71).

Melatonin [Circadin®] and other analogues, non-selective MT1/MT2 receptor ligands, are presently used in therapeutically conditions. For instance, ramelteon [Rozerem®] in insomnia, agomelatine [Valdoxan®] in depression and tasimelteon [Rozerem®] in non-24-h sleep-wake disorder (3). Melatonin and related analogues also find therapeutic actions in jet lag, shift work and others circadian disorders, depressive disorders, dementia and neurologic diseases, as well as in breast and prostate cancers (3) Non-selective MT receptor agonists also include 6-chloromelatonin, 6-

hydroxymelatonin, 2-iodomelatonin, GR 196429, UCM 793 and 2-methoxy-α,β-didehydro-agomelatine (23).

Melatonin treatment in humans showed a strong safety profile and only mild adverse effects, such as headache, dizziness, nausea and sleepiness, without any serious adverse effect both with short or long-term use (134).

Despite these evidences in favor of melatonin, a recent placebo-controlled single-blind study including 21 healthy women reported that 5 mg of melatonin decreased glucose tolerance, both in the morning and evening, by decreasing insulin release and insulin sensitivity, respectively. (135). Furthermore, a common genetic variant associated with increased T2DM risk, MTNR1B rs10830963, was related with worst glucose tolerance in response to melatonin administration (136). More studies are needed to clarify this controversy. Additionally, highly potent and selective ML agonists, including tasimelteon, ramelteon, piromelatin and IK7 have been used in sleep disorders. However, there is no sufficient clinical support for the exact influence of these drugs on glucose homeostasis, insulin secretion and diabetes risk (43). More research exploring the effects of exogenous melatonin and its potential agonists on glucose metabolism is required.

CLINICAL IMPLICATIONS

Melatonin seems a potent additional treatment in glucose homeostasis, preventing from glucose intolerance, diabetes and associated comorbidities. These effects are especially due to its central capacity to reset central clock, while coordinating peripheral clock machinery. These effects are especially important when referring to people forced to timing disruption as exposure to light during night, shift work, social and jet lag, as other concerns in nowadays society, especially when other metabolic disturbances are involved. Therefore, this clock dysfunction may contribute to the emerging worldwide prevalence of T2DM. A vicious circle between these conditions appears highly probable. Indeed, diabetes may lead to sleep disorders and consequently to rhythmic disturbances that can interfere with melatonin dysregulation patterns and exacerbate this process. Considering the importance of adequate timing of food intake, exercise, adequate sleep habits and exposure to light, we highly support patients under irregular schedules should be advised to improve or maintain metabolic health. Finally, it is important to clarify the putative role of melatonin in preventing diabetes in pre-diabetic patients, mainly when circadian disruption factors are present, as in the protection from circadian disruption when diabetes is already established. One of the next steps could also be to evaluate rational combinations of melatonin or its agonists with other antidiabetic therapeutic agents.

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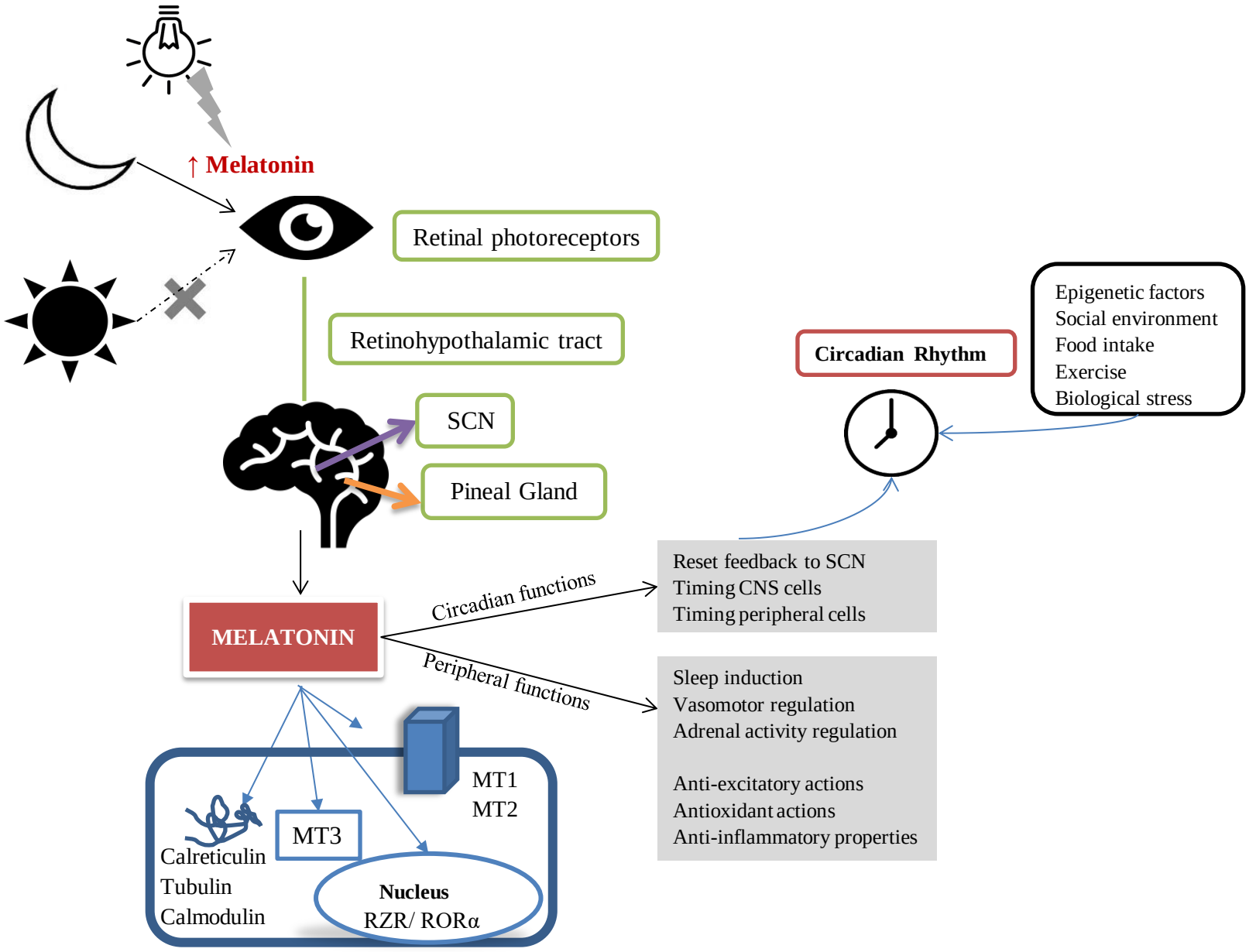
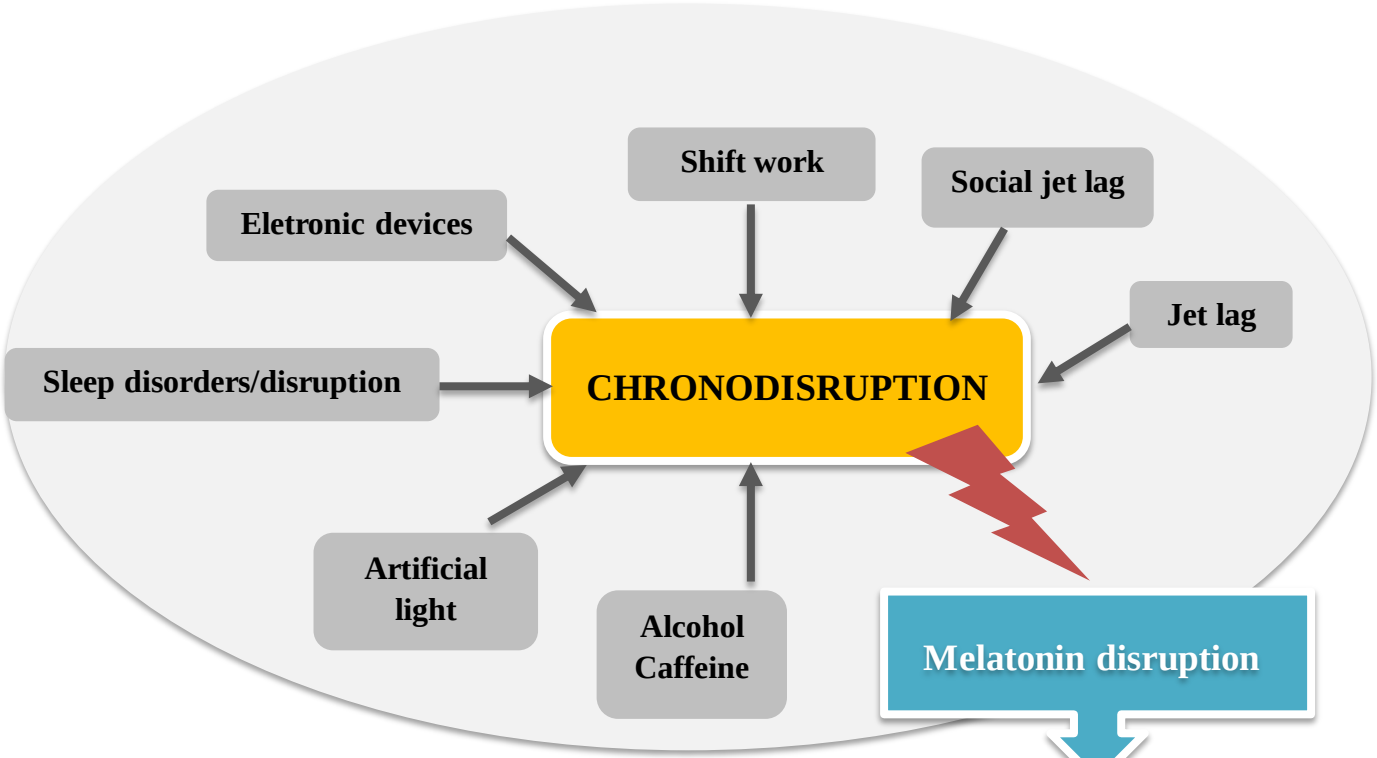
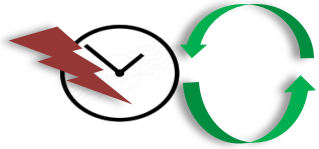

↑ Melatonin
Retinal photoreceptors
Retinohypothalamic tract
SCN
Pineal Gland
MELATONIN
Circadian functions
Peripheral functions
Reset feedback to SCN
Timing CNS cells
Timing peripheral cells
Sleep induction
Vasomotor regulation
Adrenal activity regulation
Anti-excitatory actions
Antioxidant actions
Anti-inflammatory properties
MT1
MT2
MT3
Calreticulin
Tubulin
Calmodulin
Nucleus
RZR/ RORα
Circadian Rhythm
Epigenetic factors
Social environment
Food intake
Exercise
Biological stress

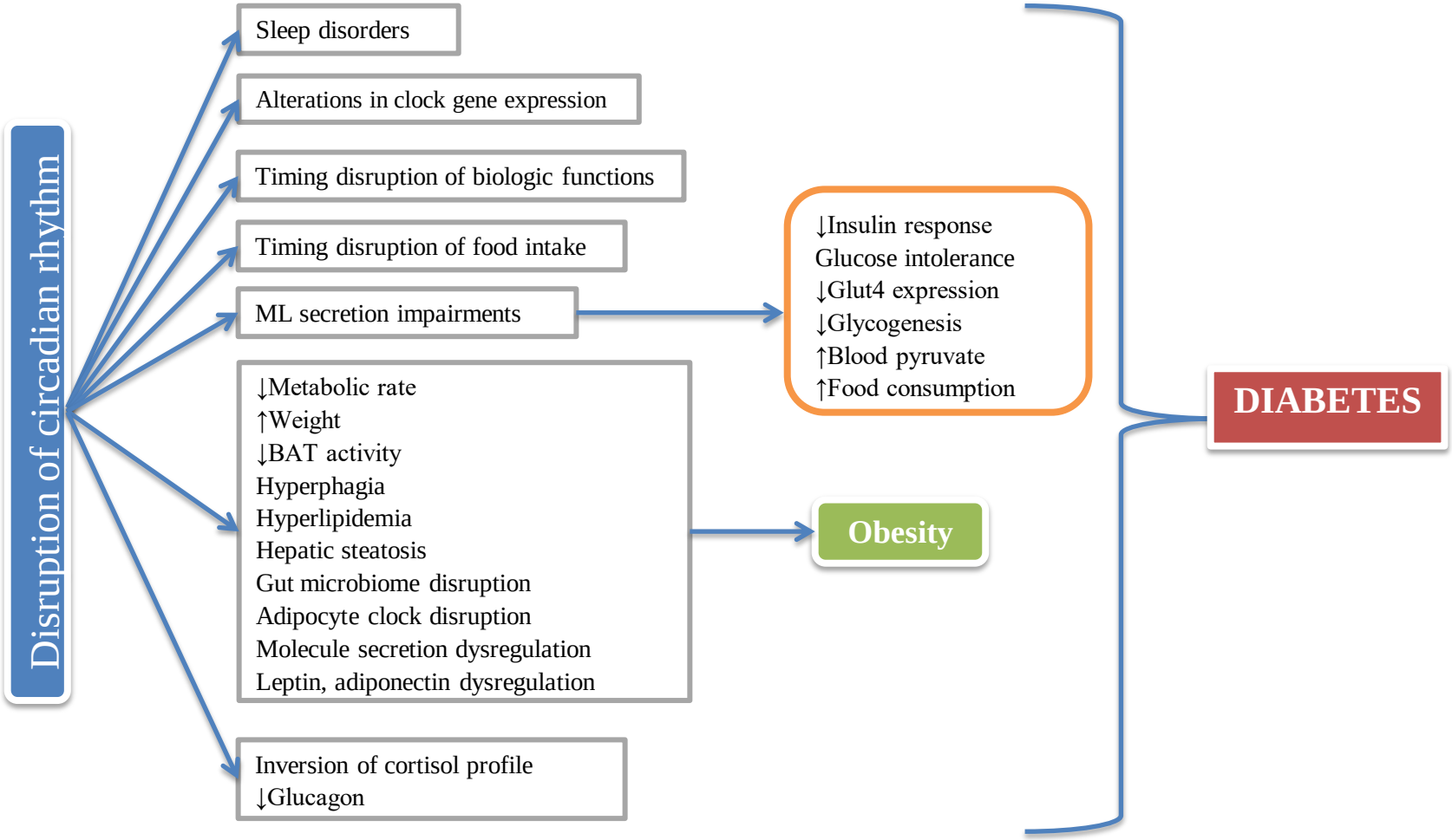
Figure 1: During the night, Melatonin (ML) is released from pineal gland following a circadian rhythm controlled by the “master circadian clock” on suprachiasmatic nucleus (SCN). ML acts mainly through MT1 and MT2, two high-affinity G protein-coupled receptors. ML can also exerts its functions binding to a third ML receptor, previously defined as MT3, a member of the quinone reductases family, through nuclear receptors, called retinoid-related orphan nuclear hormone receptor from RZR/RORα family and through direct interactions with intracellular proteins, including calmodulin, calreticulin and tubulin. ML influences circadian biologic rhythm and has important functions in other peripheral organs and cells. Simultaneously, other stimuli have impact on circadian clock, as epigenetic factors, social environment, food intake, exercise and biological stress. Natural, or artificial light, is detected by retinal photoreceptors and action potentials are transferred, via retinohypothalamic tract, to the SCN, situated in the ventral part of the hypothalamus, leading to ML synthesis suppression.





Metabolic disorders	Cardiovascular consequences	Cancer
Glucose intolerance Insulin resistance Obesity Dyslipidemia Metabolic syndrome	Coronary disease Myocardial infarction Cerebrovascular accident	Breast Prostate Colon

- Figure 2: Factors such as shift work, “jet lag” due to travels across several time zones, “social jet lag” in a context of changes in sleep behavior, sleep disorders, exposition to nocturnal lighting, mainly due to electronic devices use, and caffeine and alcohol consume have been associated to chronodisruption and consequent impairment of melatonin secretion normal patterns. This imbalance leads to cardiovascular consequences as coronary disease, myocardial infarction and cerebrovascular accident, to increase the risk of some cancer types, particularly breast, colon and prostate, and to metabolic disorders, as glucose intolerance, insulin resistance, obesity, dyslipidemia and metabolic syndrome. These last conditions might in turn be able to disturb the circadian clock in other organs and consequently to exacerbate metabolic disease.


Disruption of circadian rhythm
Sleep disorders
Alterations in clock gene expression
Timing disruption of biologic functions
Timing disruption of food intake
ML secretion impairments
↓Insulin response
Glucose intolerance
↓Glut4 expression
↓Glycogenesis
↑Blood pyruvate
↑Food consumption
↓Metabolic rate
↑Weight
↓BAT activity
Hyperphagia
Hyperlipidemia
Hepatic steatosis
Gut microbiome disruption
Adipocyte clock disruption
Molecule secretion dysregulation
Leptin, adiponectin dysregulation
Obesity
Inversion of cortisol profile
↓Glucagon
DIABETES

- Figure 3: Disruption of circadian rhythm (chronodisruption) is implicated in diabetes pathogenesis. Its mechanisms might be related with sleep disorders, alterations in clock gene expression and timing disruption of biologic functions and food intake. Leading to melatonin (ML) secretion impairments, disruption of circadian rhythm can also result in a decrease in insulin response, Glut4 expression and hepatic and muscular glycogenesis, increase in blood pyruvate concentrations and food consumption, and glucose intolerance, contributing to diabetes development. Other circadian disruption consequences are responsible for obesity pathogenesis, a main risk factor for type 2 diabetes mellitus (T2DM), as decreased metabolic rate, weight gain, decreased brown adipose tissue (BAT) activity, which has protective role in diabetes and in glucose homeostasis, hyperphagia, hyperlipidemia, hepatic steatosis and gut microbiome, adipocyte clock, molecules and hormones (leptin and adiponectin) secretion dysregulation. Inversion of cortisol profile and a decrease in glucagon levels, both consequences of chronodisruption, influence negatively glucose homeostasis and consequently increase T2DM risk.

Anexos

1. **Normas da revista: Hormones**

INSTRUCTIONS TO AUTHORS

INSTRUCTIONS FOR PREPARATION OF MANUSCRIPT

HORMONES publishes articles related to:

- Original research findings on humans or experimental animals
- Translational research
- Complete reviews of the Literature
- Case reports will exceptionally be considered only when they present some novel mechanism or previously unknown mutation etc
- Editorial Commentaries
- News and Views
- Book Reviews
- Letters to the Editor
- Short notes on etymology of medical terms
- History of Endocrinology

PREPARATION OF MANUSCRIPTS

- Must be written in correct English.
- Use wide margins
- Number all pages
- Use separate pages for title page, references, footnotes, tables, legends
- Figures should be created with high resolution software

The title page should include:

- Full title
- Abbreviated title of not more than 40 characters
- Authors' names and institution affiliations
- Corresponding author's address, telephone number, fax and e-mail
- Key words up to 8
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ABSTRACT

For research papers the abstract must be structured: Objective , Design, Results, Conclusions. It should not exceed 250 words. An abstract is not necessary for review articles.
Describe briefly the background, the aim of the study or the hypothesis tested, the methods used, the results and the conclusions.

MAIN TEXT

Follow the usual architectural model, namely: introduction, subjects or experimental animals and methodology, results, discussion.
Laboratory values should be stated in both the international system (SI units) and in metric units in parenthesis. Values in the abstract values should be given only in SI units. Temperatures should be written in degrees Celsius.

FOOTNOTES

Footnotes should be avoided. When considered necessary they should be numbered consecutively and placed at the foot of the appropriate page.

FIGURES

For electronic submission use a separate file for each figure in one of the three acceptable formats TIF, EPS, JPG (at least 300dpi). Figure legends should contain all the information necessary without

reference to the text. Number in Arabic numericals.

TABLES
Should be presented on separate pages as printed text. Tables require a concise heading.

REFERENCES
References should be cited consecutively in numerical order in the text (as superscript outside the punctuation) and the same numerical order must be followed on a separate sheet at the end of the manuscript. The title of the Journals used follow the abbreviated style used in the index medicus. The author is responsible for the accuracy of references.
Examples
• Papers published in Journals:
Mahagan T, Lightman SL, 2000 A simple test for growth hormone deficiency in adults. J Clin Endocrinol Metab 85: 1473-1476.
When the number of authors exceeds six, the designation et al must be used after the third author.
• Books:
Mazzaferri EL 1993 Thyroid carcinoma. Papillary and follicular. In: Mazzaferri EL, Samaan N (eds) Endocrine tumors, Blackwell Scientific Publications Inc, Cambridge; pp, 278-333.

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ETHICAL CONSIDERATIONS
Pulished research must comply with the guidelines for human studies and animal welfare regulations. Authors should state these, when applicable. The relevant statement should be accompanied by a notation of approval by the institutional committee on human research in the Materials and Methods section.

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